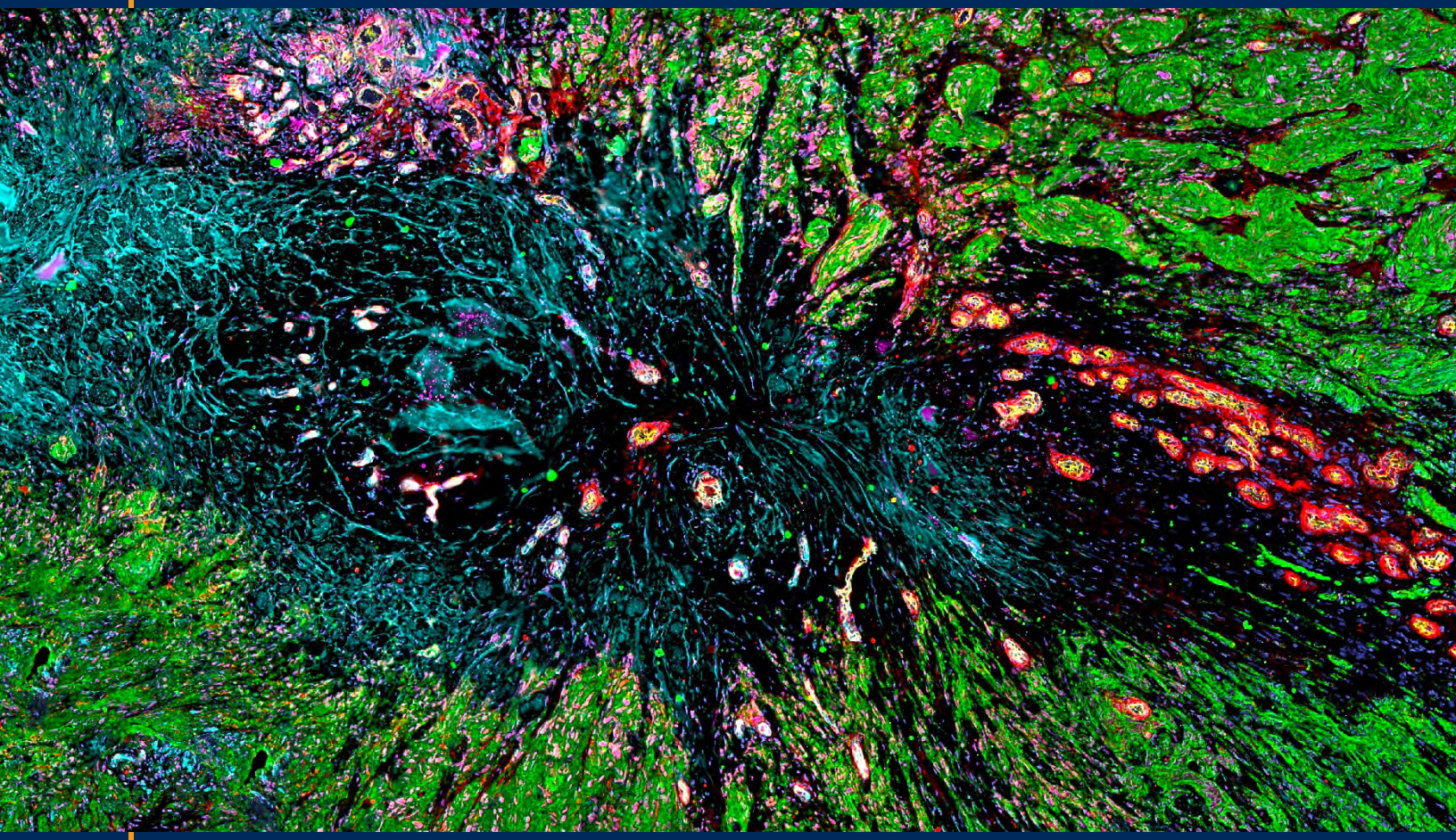
December 2024



# Ludwig Link




LUDWIG CANCER RESEARCH

LIFE-CHANGING SCIENCE

# Welcome

**Unmesh Kher**
Editorial Director

Here it is: Our December issue of the Ludwig Link!

You'll find in these pages the usual smorgasbord of research briefs from across the Ludwig community, including reports on a remote-controlled CAR-T construct, how certain T cells keep metabolism in tune and on time, new strategies for treating triple-negative breast cancer and novel markers to select patients likely to respond to TIL-ACT therapy.

We also have news reports on a program launched this year focused on myeloid cells in cancer, and on a very successful scientific retreat the Ludwig Institute recently convened in Portugal.

A couple of issues ago, we began what we plan to make a regular feature in the Ludwig Link—interviews with trainees across our Branches and Centers. You'll see in this issue our second set of interviews with some of Ludwig's bright early-career scientists—one each from Ludwig Harvard, Ludwig Johns Hopkins and Ludwig Stanford. Additionally, we introduce you to the newest PI in our community—Bethan Psaila, who officially joined Ludwig Oxford this past fall.

Finally, you might have noticed that we've developed a minor obsession with pictures of the usual protagonists of our stories—cells, cancerous and otherwise. There may be dutiful reasons to run them in the Link, but the truth is we just can't resist a sexy micrograph. To say we're pleased that the multiplex tints leap from the pages of this issue would be a bit of an understatement.

We mention this—but of course—because we have an ask: If any of you have pretty micrographs you'd like to show off to the world, we're happy to help you do so. Please send them our way and, with your permission, we'll post them as appropriate on our website, social media and perhaps even randomly in this magazine whenever we feel we'd like a little extra color.

With that, I leave you to peruse this issue.

Happy reading!

Unmesh

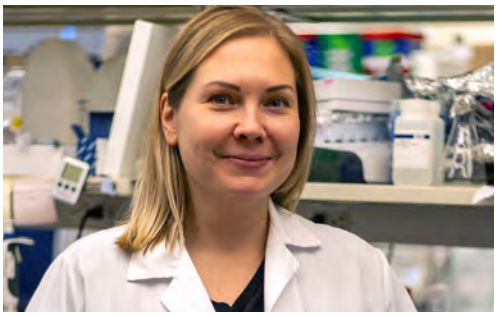
**On the cover**

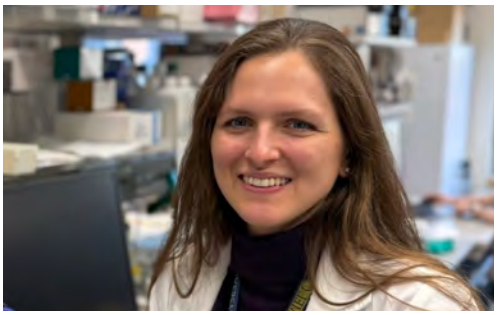
Hyperplexed image of a glioblastoma fibrotic core, caused by treatment of the primary tumor. Cells in green are recurrent tumor growth emanating from the core. Researchers led by Ludwig Lausanne's Johanna Joyce and alumni Spencer Watson and Anoek Zomer reported in *Cancer Cell* in September that recurrent glioblastoma multiforme tumors can grow out of the fibrous scars left by the treatment of the initial tumor in mice as well as humans. Studies in mice revealed that the scars create a niche that shields residual glioma cells from immunosurveillance and pushes them into a dormant state in which they resist therapy. Image by Spencer Watson.

STORY ON PAGE 21

# In this issue

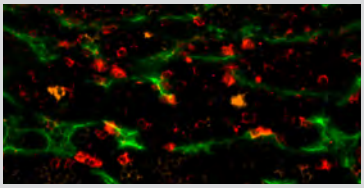








**FEATURED RESEARCH**



Ludwig MIT's Stefani Spranger and colleagues discovered in the white pulp of the spleen a subpopulation of T cells in a state of intermediate exhaustion whose descendants contribute enormously to anti-tumor responses following immune checkpoint blockade therapy. **PAGE 19**

Marcia Haigis

David Pellman

G.Weber / CHUV

George Coukos

Matthew Vander Heiden

# Four Ludwig researchers join the National Academy of Medicine

Ludwig Harvard's Marcia Haigis and David Pellman, Ludwig Lausanne Director George Coukos and Ludwig MIT's Matthew Vander Heiden were elected in October to the U.S. National Academy of Medicine (NAM). They join a class of 90 regular and ten international members inducted into the Academy this year. Election to the NAM, one of the highest honors bestowed on professionals for their contributions to biomedical disciplines, recognizes "outstanding professional achievement and commitment to service." In naming George to its membership, the NAM cited his landmark discovery of "the correlation between T-cell infiltration and favorable prognosis in ovarian cancer, an observation later generalized to all human tumors." The Academy also noted George's subsequent work revealing how the blood vessels that feed tumors serve as an active barrier to anti-tumor T cells, and his demonstration that therapies that normalize the tumor vasculature can drive T cell infiltration and fuel the immune rejection of malignancies. This work has spawned therapeutic strategies combining anti-angiogenic drugs with immunotherapies. Marcia and Matthew were both cited by the NAM for their pioneering studies of cellular metabolism. Marcia was especially noted for "elucidating how metabolites contribute to normal physiology, aging, cancer, and anti-tumor immune control." Her discoveries, the Academy observed, have "informed how diet and age alter metabolite interactions, leading to disease." Matthew was recognized for his studies exploring "how [cell metabolism] influences cancer initiation, progression, and therapy responses." The NAM added that his "work has contributed to the development of approved therapies for cancer and anemia," and that he is a "thought leader in understanding metabolic phenotypes and their relations to disease pathogenesis." David, meanwhile, was honored "for identifying the mechanistic basis for mutational processes that generate a large fraction of the structural and numerical chromosome abnormalities in cancer and certain congenital diseases." The NAM additionally mentioned "his discovery of a mechanism explaining chromothripsis," or the shattering and aberrant reassembly of chromosomes, which it said "is considered a landmark in cancer genetics." We extend our congratulations to all four of our colleagues for this well-deserved recognition of their contributions to cancer research and human biology.

# A Ludwig Lausanne collaboration takes aim at myeloid cells in cancer

Myeloid cells of the immune system—like macrophages, dendritic cells and neutrophils—play many and often paradoxical roles in the tumor microenvironment (TME). Macrophages, for example, can gobble up malignant cells and, like dendritic cells, orchestrate a T cell assault on tumors. Or they can suppress immune responses, nurture the growth of cancer cells and abet their metastasis. Similarly, neutrophils can be assets to immunotherapy. But they can just as easily be manipulated by the TME to shield cancer cells from immune attack or build a niche for their metastatic progeny.

In other words, wherever something interesting is happening in a tumor—from metabolic shenanigans to stromal remodeling—you're likely to find a myeloid cell in the mix. If this makes for some fascinating biology, it also raises the profile of these multifaceted cells as prospective stars in an array of possible immunotherapies. And few places are better equipped to explore that possibility than the Ludwig Lausanne Branch, which has emerged as a global hotspot of research on myeloid cells in tumor biology. To better harness this potential, the Ludwig Institute for Cancer Research invested in a collaborative discovery and translational research program at the Branch called the Myeloid Cells in Cancer Initiative (MCCI). Notably, the Initiative leverages the Branch structure of the Ludwig Institute, which was devised by the Institute's architects to engender scientific collaboration. Rather than focusing on individual labs or scientific stars—as do many other biomedical research funders—Ludwig's Branches naturally enable the pooling of multiple disciplines and talents to solve important problems in cancer biology.

The ultimate goal of the MCCI is to create a cancer-associated myeloid cell atlas and advance a set of pioneering therapeutic agents into human trials. Its immediate objectives are equally well defined. Participating laboratories will, for starters, identify key genes that orchestrate the pro-tumoral functions of myeloid cells that are also dispensable in healthy tissues and can thus be safely targeted for cancer therapy. A handful of such genes have already been identified by the MCCI's participating laboratories. The researchers will also identify unique genetic payloads, such as cytokines and antibodies, that can be engineered into myeloid cell progenitors to undermine various pro-tumoral functions. Why progenitors? Because, unlike their mature descendants, these cells are easily expanded in culture and persist far longer in both labs and living animals. Finally, the Initiative will pin down gene promoters and enhancers that can be used to ensure the targeted expression of those payloads in the tumor myeloid compartment and not in healthy tissues, where such expression could prove toxic.

The MCCI is divided into two main arms (see next page). A Discovery Program, primarily involving the laboratories of Doug Hanahan, Ping-Chih Ho, Johanna Joyce, Mikaël Pittet and Bernhard Gentner, explores novel myeloid targets that are both readily actionable and amenable to clinical translation. A program led by Mikaël is also charting myeloid cell states across multiple cancers in mouse models and humans to generate a reference atlas for use by the MCCI as well as other laboratories across the Ludwig community—and beyond. Its aims are to define similarities and differences between myeloid cells and their states across malignancies to identify how they influence clinical outcomes, and to determine which mouse models are optimal for the study of cancer-associated myeloid biology.

A Translational Program led by Bernhard and Ludwig Lausanne Director George Coukos will generate and preclinically evaluate products that flow out of the Discovery Program and develop manufacturing processes to smooth their path into human trials.

Given the talent amassed behind the effort, it's a fair bet more than a few will complete that journey.

Douglas Hanahan

Johanna Joyce

Ping-Chih Ho

Mikaël Pittet

Bernhard Gentner

George Coukos

G.Weber / CHUV

## Initiative rationale

Myeloid cells are linked to every hallmark of cancer but remain relatively untapped immunotherapy targets.

### Bottlenecks

- Which myeloid cell states are most relevant targets?
- How to interfere with them in therapeutic settings?

### Strengths of Ludwig Lausanne Branch

- Unparalleled expertise in myeloid cell biology
- Ability to translate findings through cell therapy interventions

### Objectives

- Combine our skills to reverse myeloid cell suppression through cell engineering and cell transfer
- Advance the most promising compound(s) into the clinic

## Discovery program

### Goals

1. Reveal new myeloid targets
2. Generate and validate engineered progenitors
3. Define the therapeutic potency of engineered progenitors in mice

## Myeloid atlas project

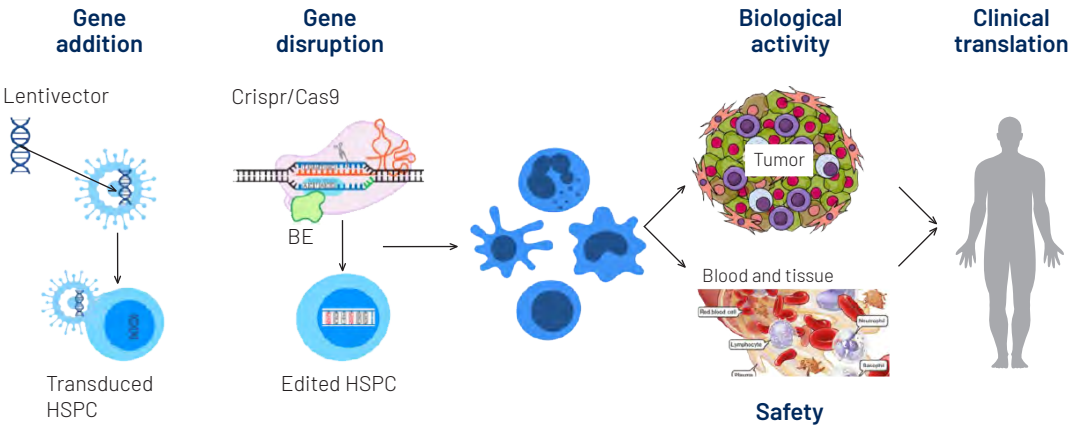
Charts myeloid cells and their states across multiple cancers in mouse models and humans to generate a reference atlas for the field

## Translational program

### Goals

1. Generate engineered human myeloid progenitors
2. Test safety and biological activity for the lead targets
3. Develop a manufacturing process for clinical testing

### Approaches used to produce and test the engineered human cells



Retreat attendees after a sumptuous lunch in bright sunshine and (literal) round-table discussions on issues of importance the field.

# A fall meeting in Sintra

Folks from the far-flung labs and administrative offices of the Ludwig Institute for Cancer Research converged on Portugal in late September for a scientific retreat in the historic municipality of Sintra, on the outskirts of Lisbon—or rather, Lisboa, as tour guides pointedly reminded attendees. Board members, scientific advisors and a few delightful guests from other institutions also attended the retreat, which doubled as a celebration of the careers of Chairman-emeritus John Notter and his replacement as Chair of the Board, former Ludwig Institute CEO and President Edward McDermott. Lively scientific discussions unspooled in both structured and unstructured events over a couple of days. Organized by a planning committee led by Ludwig Institute Deputy Scientific Director and Communications Director Pat Morin and powered by the efforts of Tahmid Shahid and Jennifer Bencivenga of the New York office, the retreat's judiciously conceived agenda included brief presentations by Branch directors and flash talks by Assistant and Associate Members on their research. It also featured a panel discussion with the Institute's scientific advisors and another on the history of the Institute, accompanied by the formal launch of a book on that topic published by Ludwig Communications. And there were group discussions—held over lunch outdoors in bright sunshine—on matters of general relevance to the field such as artificial intelligence in cancer research, scientific integrity and overcoming barriers to interinstitutional collaboration, among other things. Many attendees met their Ludwig colleagues in the flesh for the first time, discovering areas of shared interest and making new friends. Indeed, the informal discussions over meals and other leisure activities proved to be a much-appreciated feature of the retreat, as they opened doors to new collaborations across Ludwig Branches. Many attendees mentioned we really should do this sort of thing more often. The Link couldn't agree more.

Ludwig Institute Scientific Director and CEO Chi Van Dang, right, with Ludwig Oxford's Yang Shi.

Lydia Lynch

Rhythmic IL-17 production by γδ T cells maintains adipose de novo lipogenesis | *Nature*, 2024 October 30

## How γδ T cells keep fat metabolism in tune and on time

Researchers led by Ludwig Princeton's Lydia Lynch reported in a *Nature* paper in October that innate IL-17-producing T cells—including γδ T cells, invariant natural killer T cells and mucosal-associated invariant T cells—express clock genes, which coordinate cellular physiology with the circadian cycle, at especially high levels. The immune system follows circadian rhythms to better combat potential infections, but these patterns have also been shown to be critical to its role in maintaining tissue health and integrity. Lydia and her team discovered that IL-17-producing γδ T cells, which reside in fat, rely on the molecular clock to maintain fat tissue homeostasis. These immune cells make the proinflammatory cytokines IL-17A and IL-17F in a rhythmic pattern that peaks at night, even in the absence of infection. They show that this is because rhythmic production of IL-17 is required to maintain fat metabolic rhythms, and that the cytokine is required for de novo lipogenesis—or the production of fat from sugar—in fat tissue. A lack of IL-17 disrupts circadian body temperature regulation (leaving mice cold) and the schedule on which sugars or fats are burned for energy. The findings of this study also have relevance for cancer, as mouse models that lack genes for IL17A/F have slower growing tumors. They suggest this could be due to IL-17's influence on the lipid metabolism of tumor cells.

**People on the move**

## Bethan Psaila joins Ludwig Oxford as an Associate Member

Bethan Psaila

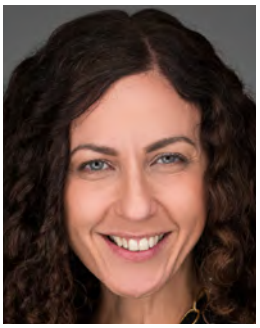
Clinician-scientist Bethan Psaila joined Ludwig Oxford as an associate member in the fall, bringing to the Branch her considerable expertise on myeloproliferative neoplasms (MPNs), slow-growing blood cancers that originate in the bone marrow. An Associate Professor of Hematology at the University of Oxford, Beth also sees patients at the Oxford University Hospitals NHS Trust, where she is a regional specialist caring for MPN patients and leads clinical trials focusing on novel immunotherapies for these cancers. Beth's lab explores the interactions between blood stem cells, megakaryocytes/platelets and the bone marrow stroma in both the healthy generation of blood cells (hematopoiesis) and in cancers. Its work focuses on studying primary samples from patients and complex human tissue models (organoids) to discover and validate new strategies to selectively target the cancer clone and prevent or treat progression to myelofibrosis—an advanced form of MPN that has a median survival of only 5-7 years following diagnosis. Beth and her team also explore intriguing aspects of megakaryocyte and platelet biology—for example, how megakaryocytes develop and tolerate multiple copies of their nuclear genome and the utility of platelets for liquid biopsies—and its implications for human cancers. Prior to Oxford, Beth trained at Clare College, Cambridge, Imperial College London/The Hammersmith Hospital, Cornell Medical College in New York and the National Institutes of Health in Bethesda, Maryland.

# A potential strategy to treat the deadliest of breast cancers

Karen Cichowski

**AKT and EZH2 inhibitors kill TNBCs by hijacking mechanisms of involution** | *Nature*, 2024 October 9

Some 70% of triple negative breast cancers (TNBCs)—the deadliest subtype of breast malignancies—have alterations in PI3 kinase signaling pathway members PIK3CA, AKT1 or PTEN. Researchers led by Ludwig Harvard's Karen Cichowski reported in an October publication in *Nature* that a combination of two therapeutic agents, one of which targets this pathway, could drive TNBC cells into a distinctly treatable state. They discovered that AKT inhibitors synergize with agents that suppress the histone methyltransferase EZH2 to drive robust tumor regression in multiple mouse models of TNBC, including those derived from patient tumors. The inhibitors exert these effects by cooperatively driving basal-like TNBC cells into a more differentiated, luminal-like state. (Basal cells help circulate milk during lactation, while luminal cells in mammary glands produce milk.) This differentiation cannot be induced by either agent alone. Once TNBCs are differentiated, the agents kill them by hijacking signals that normally drive mammary gland involution, a naturally occurring process in which milk-producing cells die after the cessation of lactation. The researchers also used machine learning to predict patient responses to this strategy, which could help set the stage for clinical trials. Karen and her colleagues note that their findings illustrate how deregulated epigenetic enzymes can protect tumor cells from oncogenic vulnerabilities and how developmental cell death pathways unique to specific tissues might be exploited for cancer therapy.



Galit Lahav

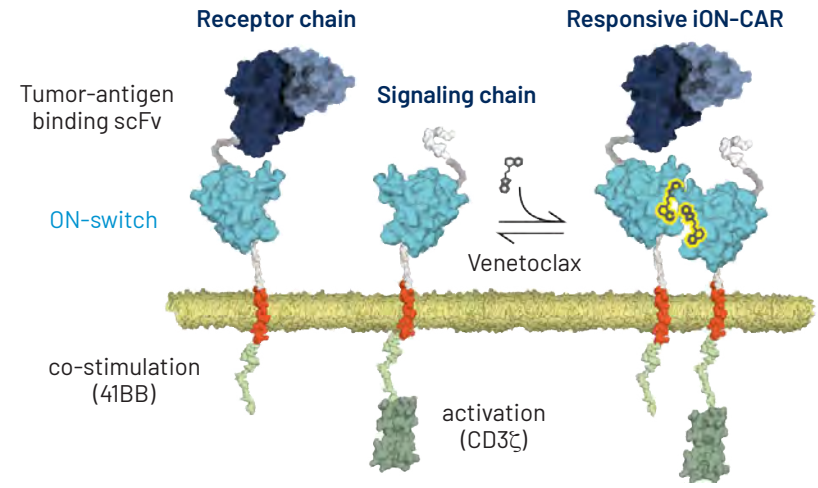
**Temporal regulation of gene expression through integration of p53 dynamics and modifications** | *Science Advances*, 2024 October 25

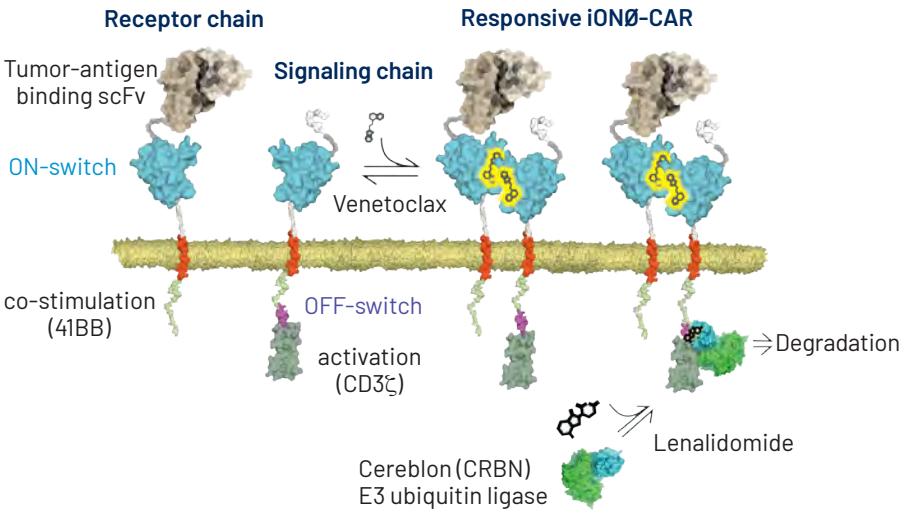
# How the guardian of the genome times its multiple tasks

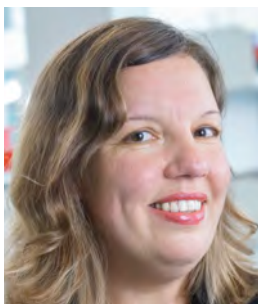
One of the most frequently mutated genes in cancer, the transcription factor p53—popularly known as the Guardian of the Genome—orchestrates a multitude of vital intracellular processes in response to DNA damage, such as DNA repair, cell cycle arrest and apoptotic cell suicide. Post-translational chemical modifications tacked on to the transcription factor are known to play a central role in determining which of various gene expression programs it initiates in any given circumstance. It is also known that cellular levels of p53 oscillate in response to double-stranded breaks to DNA. Less clear is how p53 temporally coordinates downstream cellular responses with its pulsed expression. Researchers led by Ludwig Harvard's Galit Lahav showed in *Science Advances* in October that the protein is differentially modified in its first and second pulses of expression. Acetylation at two sites of the protein, K373 and K382, is reduced in the second phase of p53 expression following double stranded DNA breaks. Galit and her colleagues show that the reduced acetylation in the second pulse alters the menu of gene expression programs p53 activates. This suggests that changing patterns of post-translational modification may enable cellular responses to p53-activating stimuli to evolve over time. The interplay of pulsed expression and chemical modification might permit p53 to temporally organize multiple processes in individual cells.

# A remote-controlled CAR-T therapy

Chimeric antigen-receptor (CAR) T cell therapies face two major challenges when deployed against solid tumors. First, many solid tumor antigens are also found at low levels on healthy cells, raising the risks of so-called "off-tumor, on-target" effects. Second, the immunosuppressive conditions of the solid tumor microenvironment can push CAR-T cells into a state of dysfunction known as "exhaustion". A team led by Ludwig Lausanne's Melita Irving and Greta Maria Paola Giordano Attianese reported in an October *PNAS* paper the design and evaluation of new types of CAR-T cells that could address both these challenges. Named "inducible-ON" (iON) CAR and iON/OFF CAR (iONØ-CAR), the cells can be switched on to varying degrees of intensity and then switched off on demand—to stem toxicity—using drugs that are already employed in the clinic. The iON CAR was made by separating the antigen-binding moiety of the CAR and the internal, signaling component of the construct that is required for T cell activation on two separate chains. The two chains were also engineered with an on-switch to enable the cancer drug venetoclax to draw them together and create an active CAR complex. The iONØ-CAR, meanwhile, was made by adding a second component to the signaling chain that is bound by another cancer drug, lenalidomide. That binding marks the chain for intracellular degradation to rapidly switch off CAR-T cell activity.



Melita Irving

Greta Maria Paola Giordano Attianese

**Dual ON/OFF-switch chimeric antigen receptor controlled by two clinically approved drugs** | *PNAS*, 2024 October 25

# How slow-growing MPNs turn into leukemia-like cancers

Stefan Constantinescu

*BCR::ABL1*-negative myeloproliferative neoplasms (MPNs), a family of slow-growing blood cancers, can evolve into secondary acute myeloid leukemia (sAML) or blast-phase (BP) MPN, which is a severe leukemia-like disease that is very difficult to treat. Researchers led by Ludwig Oxford's Stefan Constantinescu explored the acquired genetic aberrations and patterns of clonal evolution that underlie transformation to BP-MPN using samples taken from 33 patients over six years at three hematology units in Romania. Aside from tracing the patterns of clonal evolution leading to BP-MPN, the researchers reported in an October *Correspondence* in the *American Journal of Hematology* that epigenetic mutations are detected in nearly 73% of cases. When copy-number variations that affect chromatin-modifying epigenetic enzymes and DNA methylation were additionally taken into account, nearly 85% of patients exhibited such genomic alterations, pointing to a major role for such mutations in MPN progression to BP-MPN. Anomalies in the EZH2 gene, which encodes a histone modifying enzyme, were especially common in people whose MPNs were driven by the common JAK2 V617F mutation or had none of the known driver mutations. Mutations in the TP53 gene were present in 33% of cases, with 75% of those also carrying epigenetic alterations. Stefan and his colleagues stress the importance of regular genetic screening of patients to evaluate clonal evolution as a means to predict disease transformation.

**Dominance of mutations in epigenetic regulators and a diversity of signaling alterations in blast-phase *BCR::ABL1*-negative myeloproliferative neoplasms** | *American Journal of Hematology,* 2024 October 19

Chunxiao Song

**Absolute quantitative and base-resolution sequencing reveals comprehensive landscape of pseudouridine across the human transcriptome** | *Nature Methods*, 2024 September 30

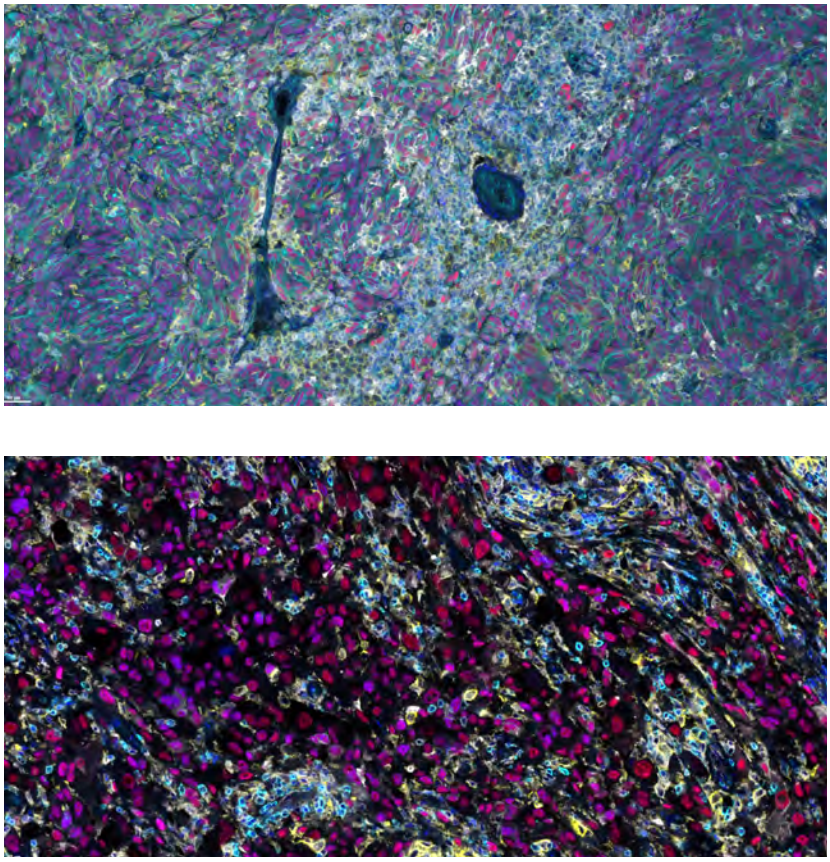
# A new standard for sequencing Ψ

Researchers are increasingly exploring the biological effects of several different kinds of chemical modifications made to RNA molecules in cells. A chemical tweak that creates a base known as pseudouridine (Ψ) is among the most common of these modifications. Its biological function has, however, not been extensively studied, in large part due to the lack of sufficiently sensitive tools for its detection. This is no longer a problem. Researchers led by Ludwig Oxford's Chunxiao Song reported in *Nature Methods* in September a method for the highly sensitive sequencing of Ψ. The technique—2-bromoacrylamide-assisted cyclization sequencing (BACS)—is based on new bromoacrylamide cyclization chemistry that induces Ψ-to-C mutation signatures for the quantification of Ψ stoichiometry and its sequencing at single-base resolution. BACS overcomes key limitations of existing methods for Ψ sequencing to generate a far more sensitive and accurate readout of the modification across RNA species. The researchers applied BACS to detect all pseudouridine sites in human ribosomal RNA. They also used their new method to generate, among other things, the first quantitative pseudouridine map of human small nucleolar RNA and transfer RNA, identify the sequence motifs and targets of three enzymes that generate the modification and an abundant pseudouridine site in Epstein–Barr virus-encoded small RNA EBER2. Chunxiao and his team expect BACS will be swiftly and widely adopted in the field for the study of pseudouridine biology.

# An AI-powered pipeline for cancer vaccine design

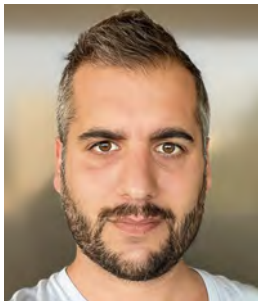
Researchers led by Ludwig Lausanne's Florian Huber and Michal Bassani-Sternberg described in an October issue of *Nature Biotechnology* a start-to-finish computational pipeline for cancer vaccine design named NeoDisc. The pipeline integrates data from genomic analyses of the tumors and normal cells from the patient as well as transcriptomics and mass spectrometry-based immunopeptidomics to generate its output. Aside from neoantigens, NeoDisc detects erroneously expressed noncoding DNA, tumor-associated genes and other aberrantly expressed gene products in cancer cells as well as viral antigens in relevant cases. It then applies both machine learning and rule-based algorithms to prioritize those most likely to elicit T cell responses and designs a personalized cancer vaccine for a patient. NeoDisc also ranks the potentially targetable antigens it detects and analyzes cancer cell heterogeneity within tumors. Further, it can detect potential defects in the machinery of antigen presentation—flagging a key mechanism of immune evasion in tumors—and thus help select patients likely to benefit from personalized immunotherapies. Michal, Florian and colleagues demonstrated that NeoDisc provides a more accurate selection of effective cancer antigens for vaccines and adoptive cell therapies than do other computational tools currently used for that purpose. The computational pipeline at the heart of NeoDisc is already being used in clinical trials of personalized cancer vaccines and adoptive cell therapies in Lausanne.

**A comprehensive proteogenomic pipeline for neoantigen discovery to advance personalized cancer immunotherapy** | *Nature Biotechnology*, 2024 October 11



Stephanie Tissot, published in *Nature Biotechnology*, 2024 October 11

Multiplex immunofluorescent staining of patient melanoma tumor tissue samples, in which NeoDisc reported both HLA loss of heterozygosity and loss of functional β2-microglobulin, a common phenomenon in cancer cells (red). Both events hinder antigen presentation, which is a common mechanism of immune evasion. The other image is of a sample in which, in contrast, cancer cells express HLA-ABC (light blue) or HLA-DR (yellow).



Florian Huber

Michal Bassani-Sternberg


Marketa Tomkova


Michael McClellan


Benjamin Schuster-Böckler


Skirmantas Kriaucionis

# The source of the genome's most common mutation

Researchers led by Ludwig Oxford's Marketa Tomkova, Michael McClellan, Benjamin Schuster-Böckler and Skirmantas Kriaucionis punctured a longstanding assumption about the source of the most common type of DNA mutation, in which cytosine (C) is swapped for a thymine (T). This mutation was thought to be primarily the result of spontaneous deamination, which is about twice as likely to happen in epigenetically methylated cytosines, which occur in genomic DNA at so-called "CpG" positions. The researchers reported in *Nature Genetics* in October that CpG to TpG mutations are instead primarily generated during DNA replication and caused by the tendency of DNA polymerase ε (Pol ε) to make copying errors at methylated CpG sites.

They developed a sensitive new sequencing technology—Polymerase Error Rate Sequencing (PER-seq)—that discerns genuine errors made by Pol ε from experimental artifacts and applied it to sequence over 130 million DNA molecules. The results revealed that a common mutant of Pol ε reproduced the error signature observed in tumors with this mutation, including elevated CpG to TpG errors at methylated sites. Even the normal Pol ε produced such mutations at seven times the rate observed for nonmethylated cytosines. The findings link the incidence of CpG to TpG mutations to cell division, explaining why these mutations tend to accumulate with age and why they vary so much in frequency across tissues and tumors.

**DNA polymerase ε produces elevated C-to-T mutations at methylated CpG dinucleotides**
*Nature Genetics*, 2024 October 9

# Disrupting a deadly feedback loop

YTHDF1, which reads the N6-methyladenosine RNA modification, has been implicated in cancer progression. Researchers led by Ludwig Chicago's Chuan He, Hua Laura Liang and Director Ralph Weichselbaum reported in a September paper in *The Journal of Clinical Investigation* that in cancer patients, radiotherapy (RT) increases YTHDF1 expression in dendritic cells (DCs) but not in other types of immune cells they examined. Elevated YTHDF1 expression in DCs is associated with poor outcomes in patients receiving RT. The Ludwig Chicago team found that loss of the RNA reader enhances the antitumor effects of ionizing radiation by boosting DC priming and activation of cytotoxic T cells across multiple mouse models of cancer. RT, it turns out, elevates YTHDF1 expression in DCs through a signaling cascade involving STING—a sensor of double-stranded DNA within cells that is activated by RT and stimulates inflammation and innate immune responses by driving the expression of interferon I (IFN-I). But higher levels of YTHDF1 then counter that anti-tumor effect by driving the degradation of STING and compromising IFN-I production. To evaluate the therapeutic implications of their discovery, the researchers devised a prototype YTHDF1-deficient DC vaccine and showed that it significantly improved the therapeutic effect of RT and radio-immunotherapy in a mouse model of melanoma.

**YTHDF1 loss in dendritic cells potentiates radiation-induced antitumor immunity via STING-dependent type I IFN production** | *The Journal of Clinical Investigation*, 2024 September 26

Chuan He

Hua Laura Liang

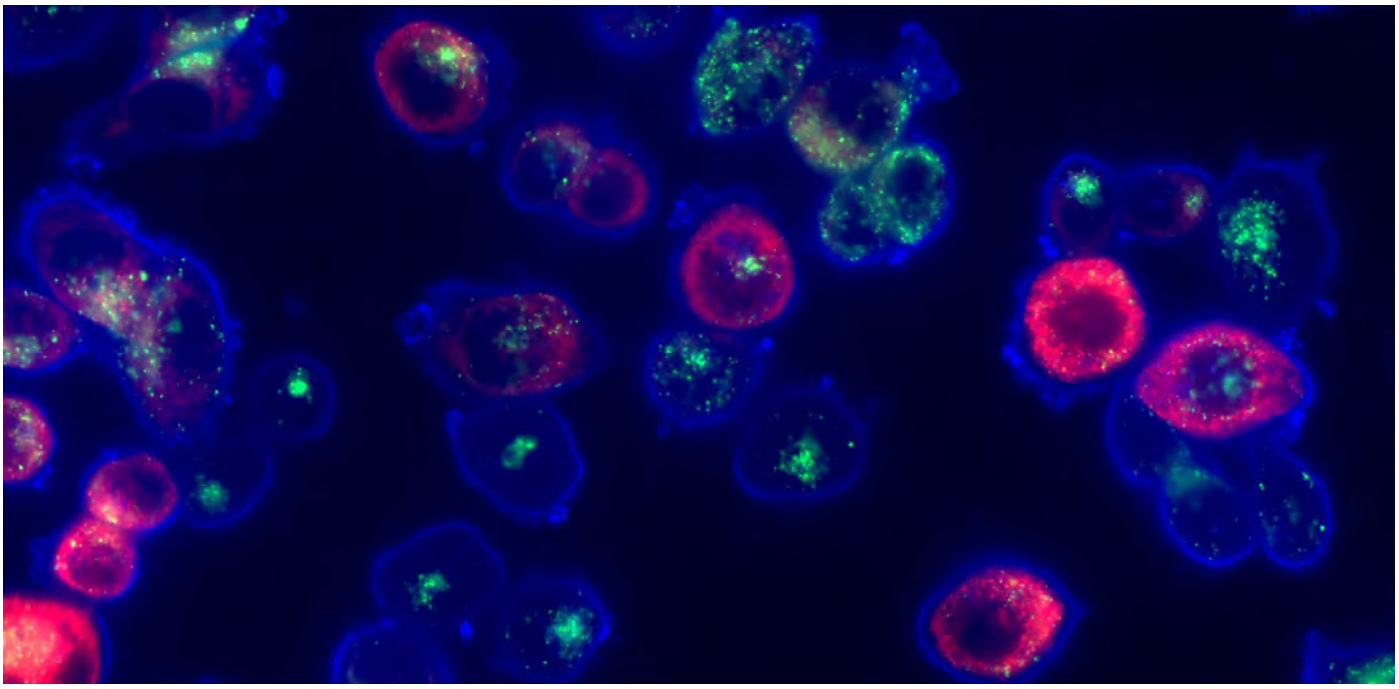
Ralph Weichselbaum

Karen Cichowski

# A two-hit take on epigenetics-based cancer therapy

Colorectal cancers are the second highest cause of cancer-related death worldwide, and nearly half of all cases are driven by mutations to the KRAS protein, which controls signaling through the RAF/MEK/ERK pathway. Drugs that target this pathway have not, however, proved effective against KRAS-mutant cancers. Further, tumor cell heterogeneity and plasticity—which is driven by epigenetic mechanisms—hamper the efficacy of targeted therapies and contribute to drug resistance in colorectal cancer (CRC). Given all this, researchers led by Ludwig Harvard's Karen Cichowski hypothesized that simultaneously targeting epigenetic and traditional oncogenic signals might push CRCs into a therapeutically vulnerable state. They reported in an August issue of *Cancer Discovery* that inhibitors of the histone methyltransferase, EZH2—an epigenetic enzyme that is overexpressed in multiple solid tumors—synergize with various RAS pathway inhibitors to induce dramatic tumor regression in mouse models of CRC. The combination, Karen and her colleagues showed, cooperatively suppresses the WNT signaling pathway, driving differentiation of CRC cells by altering their epigenetic state. The agents also induce expression of BMF, a BCL2-family protein that drives apoptotic cell death, to kill the more differentiated cells. The researchers note that their study reveals a promising strategy for treating advanced colorectal cancer, describes its mechanism of action and illustrates a paradigm for epigenetics-based cancer therapy.

**Epigenetic and oncogenic inhibitors cooperatively drive differentiation and kill KRAS-mutant colorectal cancers** | *Cancer Discovery*, 2024 August 12



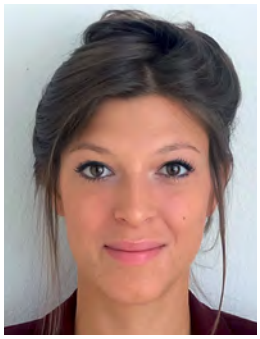
TNBC cells have low baseline lysosomal cholesterol. The image shows MDA-MB-468 triple negative breast cancer cells treated with vehicle DMSO, stained with Filipin III (blue channel, cholesterol stain), LAMP 1 (green channel, lysosome stain) and an endoplasmic-reticulum marker (red channel), overlaid.

Alex Toker

**Targeting Cholesterol Biosynthesis with Statins Synergizes with AKT Inhibitors in Triple-Negative Breast Cancer** | *Cancer Research*, 2024 October 1

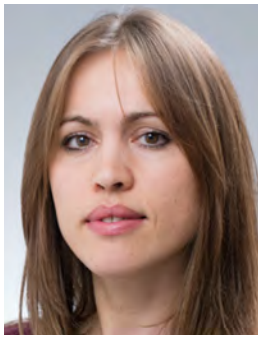
# CRISPR screen uncovers a metabolic vulnerability of TNBC

Triple-negative breast cancer (TNBC) is by far the deadliest subtype of breast malignancies. Although few effective therapies exist for TNBC treatment, dysregulation of the PI3 kinase/AKT intracellular signaling pathway is frequently seen in patients diagnosed with the cancer. Researchers led by Ludwig Harvard's Alex Toker and alumnus Alissandra Hillis conducted a genome-wide CRISPR/Cas9 screen with PI3Kα and AKT inhibitors to expose synthetic lethalities that might be exploited for TNBC therapy. They reported in an October paper in *Cancer Research* that treatment with AKT inhibitors makes TNBC cells exceptionally vulnerable to disruptions in their cholesterol balance (homeostasis) induced by a statin drug (pitavastatin). Single agent or combination treatment with the two drugs impaired activation of the transcription factor SREBP-2, which is critical to cholesterol biosynthesis and homeostasis. The researchers found that combining an FDA-approved AKT inhibitor with pitavastatin kills TNBC cells in patient-derived estrogen receptor (ER)-negative breast cancer organoids and impairs tumor growth and decreases tumor size in mice bearing xenografts of TNBC tumors. ER-positive cell lines and organoids showed no such susceptibility to the drug combination. Alex, Alissandra and colleagues suggest the findings support a clinical trial to evaluate the benefits of the drug combination for TNBC therapy, especially since both drugs involved are already in clinical use today.



Johanna Chiffelle

David Barras



Denarda Dangaj Laniti

Alexandre Harari

George Coukos

G.Weber / CHUV

# The markers of TIL-ACT efficacy

A team led by Ludwig Lausanne's Johanna Chiffelle, David Barras, Denarda Dangaj Laniti, Alexandre Harari and Director George Coukos described in a September paper in *Immunity* the correlates of efficacy for adoptive cell therapy using tumor infiltrating lymphocytes (TIL-ACT), in which T cells taken from a patient's tumors are grown in culture and reinfused for cancer therapy. Analyzing subpopulations of TILs—or clonotypes—isolated from 13 melanoma patients who received TIL-ACT in a clinical trial, the researchers detailed the origins, specificity, molecular characteristics and dynamics of T cells used for the therapy. To do so, they used single-cell RNA/TCR-sequencing, tracking TIL clonotypes from baseline tumors to ACT products and post-ACT blood and tumor samples from patients. The Ludwig Lausanne team showed that patients who benefit from the therapy have tumors rich in cancer cell-targeting TILs that expand significantly in culture and preferentially infiltrate tumors following ACT. These TILs are also transcriptionally and epigenetically reprogrammed during expansion, losing markers of exhaustion and gaining markers indicative of functional reinvigoration. Nonresponding patients have tumors devoid of tumor-reactive TILs, and their cell products are composed of large fractions of blood-borne cells. The findings should help physicians better select patients for TIL-ACT therapy and guide the design of better therapies of this kind.

**Tumor-reactive T-cell clonotype dynamics underlying clinical response to TIL therapy in melanoma**
*Immunity*, 2024 September 13

# The basic effects of acidity on T cells

Researchers led by Ludwig Lausanne's Melita Irving and senior postdoc Romain Vuillefroy de Silly reported in a September paper in *The EMBO Journal* the many ways in which the acidity of the tumor microenvironment compromises the function of cytotoxic (CD8+) T cells, which are essential to the immune clearance of tumors. The researchers discovered that acidity compromises key biochemical pathways essential to the metabolic health of CD8+ T cells as well as their responsiveness to the critical growth factor interleukin-2 (IL2). On a functional level, under acidic conditions the T cells exhibited reduced proliferation as well as impaired cytokine secretion and ability to kill target cells. Exploring the underlying mechanisms, Melita, Romain and their colleagues detailed how signaling through mTORC1—a key driver of cell growth and metabolism —is diminished in T cells under acidic conditions. Acidity additionally depresses levels of the transcription factor c-Myc, a master regulator of cellular metabolism and proliferation, due to both a decrease in its expression and an increase in its degradation. The findings underscore the potential benefits of normalizing pH in tumors in conjunction with immunotherapeutic interventions to improve patient responses.

**Acidity suppresses CD8+T-cell function by perturbing IL-2, mTORC1, and c-Myc signaling** | *The EMBO Journal*, 2024 September 16

Melita Irving

Romain Vuillefroy de Silly

Ping-Chih Ho

Alessio Bevilacqua

# A switch for T cell memory and antitumor activity

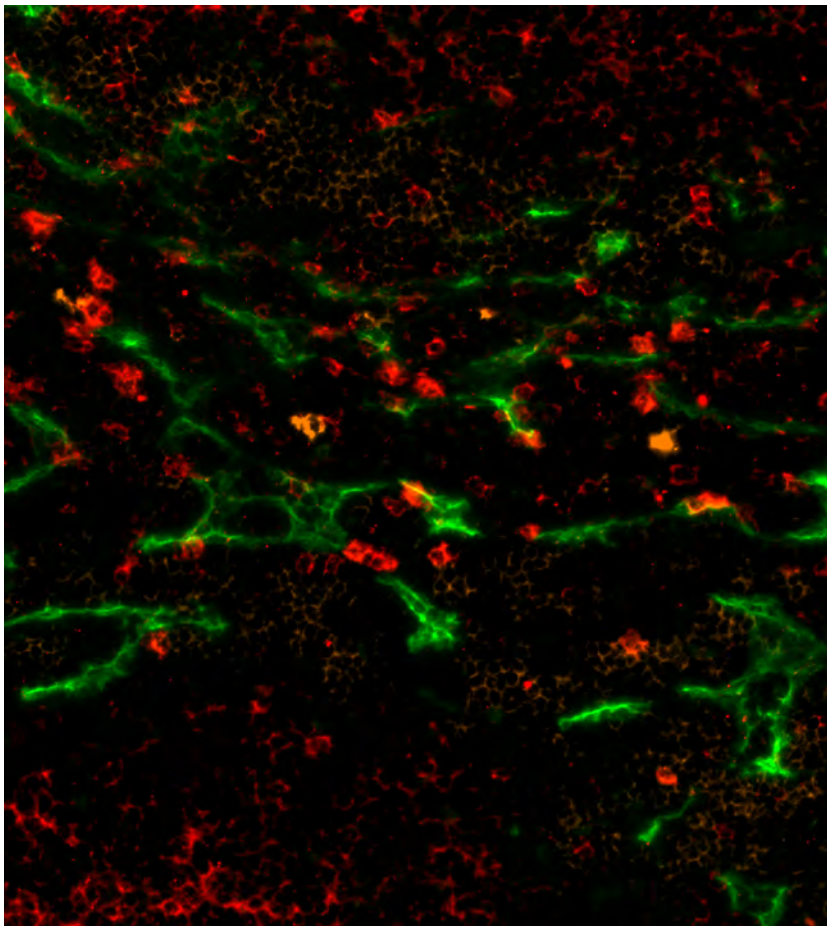
While most killer T cells involved in an immune response die off after they've done their work, a few transform into central memory CD8+ T cells (Tcms) to establish lasting immunity. Researchers led by Ludwig Lausanne's Ping-Chih Ho and Alessio Bevilacqua reported in an August issue of *Science Immunology* their discovery of a metabolic switch in T cells, PPARβ/δ, that is responsible for this transformation. A master regulator of gene expression, PPARβ/δ is activated in CD8+ T cells as immune responses wind down. The researchers show that T cell exposure to interleukin-15, which is known to support Tcm formation, and expression of a protein named TCF1 engages the PPARβ/δ pathway and helps maintain Tcms. TCF1 expression is a hallmark of a subset of CD8+ T cells—progenitor-exhausted T cells—in tumors that are activated by checkpoint blockade immunotherapy. Ping-Chih, Alessio and colleagues showed that PPARβ/δ is also essential to the formation and maintenance of progenitor-exhausted T cells. Deleting the PPARβ/δ gene from T cells led to the loss of progenitor-exhausted T cells in a mouse model of melanoma. To assess the therapeutic implications of their discovery, the researchers exposed T cells to a molecule that stimulates PPARβ/δ activity and used them to treat mice with melanoma. They showed that the cells delayed tumor growth better than their untreated counterparts, suggesting a metabolic intervention to improve cancer immunotherapy.

**PPARβ/δ-orchestrated metabolic reprogramming supports the formation and maintenance of memory CD8+ T cells** | *Science Immunology*, 2024 August 23

# The source of the ICB response

It isn't entirely clear where exactly T cells activated by immune checkpoint blockade (ICB) therapy hail from in the body or what their precise states of differentiation are at those locales. A study led by Ludwig MIT's Stefani Spranger and published in a September issue of *Science Immunology* explored these questions by profiling gene expression in individual CD8+ (or killer) T cells in tumors, draining lymph nodes and the spleen of mice treated with ICB. Stefani and her colleagues discovered a subpopulation of T cells in the white pulp of the spleen whose descendants contribute enormously to anti-tumor responses following ICB. These cells are in a state of differentiation known as intermediate exhaustion and specifically require for activation only low levels of cancer antigens in the spleen. These are presented to them by dendritic cells to generate an army of descendants that infiltrate tumors and target cancer cells in response to ICB. A lack of antigen exposure curtails their differentiation into an intermediate exhausted state, while high levels of systemic antigen push their descendants into a terminally exhausted state in which they fail to infiltrate and target tumors. The findings identify the spleen as a critical site of T cell activation in response to ICB and indicate that the density of cancer antigens within the organ plays an important role in that process.

**Expansion of tumor-reactive CD8+ T cell clonotypes occurs in the spleen in response to immune checkpoint blockade** | *Science Immunology*, 2024 September 13

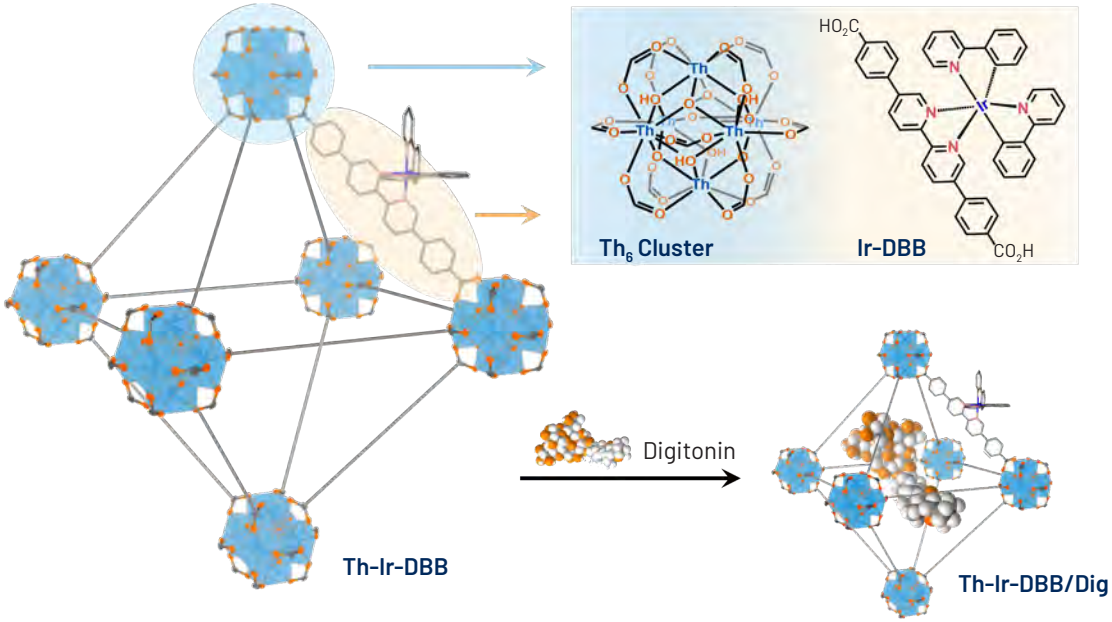


Taylor Heim

Micrograph showing T cells in the spleen, a secondary lymphoid organ. T cells are labeled red, lymphatics green. Stefani's lab has identified the spleen as a critical site of T cell activation in response to immune checkpoint blockade therapy.

Stefani Spranger

A radiotherapy-boosting, digitonin-loaded nMOF designed and preclinically evaluated by Wenbin Lin and his colleagues is constructed from heavy metal clusters (Th6), which absorb radiation with high efficiency, and photosensitizing molecules (Ir-DBB). The nMOF's components work synergistically to amplify the production of reactive oxygen species, sensitize cancer cells to radiotherapy and enhance anti-tumor immune responses.

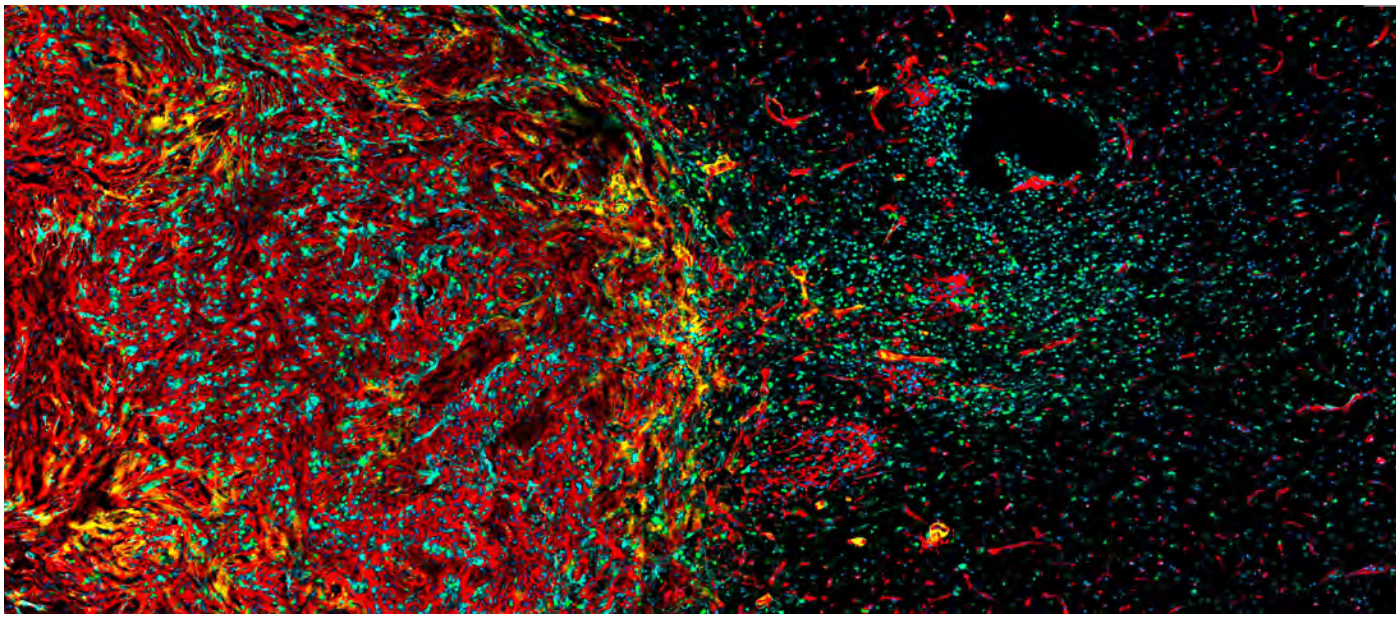



Wenbin Lin

# A tiny RT enhancer packs an outsize punch

The high energy X-rays used for radiotherapy (RT) kill cancer cells by penetrating tumors and generating highly reactive oxygen species (ROS) to damage their DNA. This destruction induces and is aided by the recruitment of immune cells to the irradiated site. The poor absorption of X-rays and generation of ROS can therefore significantly diminish the efficacy of RT, as does the frequent activation of immunosuppressive factors in the tumor microenvironment (TME). Researchers led by Ludwig Chicago's Wenbin Lin reported in a September publication in *Advanced Materials* the design and preclinical evaluation of a nanoscale metal-organic framework (nMOF) that takes on these challenges. Targeted to mitochondria, the nanoparticles—named Th-Ir-DBB/Dig, if you're wondering—are loaded with digitonin, which binds cholesterol and permeabilizes cell membranes. Its components include photosensitizing molecules that enhance ROS emission in response to even low-dose radiation. The nMOF also releases digitonin in acidic TMEs to trigger disulfidptosis—a type of programmed cell death—of cancer cells and, additionally, sensitizes them to RT-radiodynamic therapy. The released digitonin also enhances immune responses: it simultaneously downregulates multiple immune checkpoints in cancer cells (PD-L1 and CD47) and T cells (TIM3 and 2B4), mainly via cholesterol depletion. Wenbin and his team showed that low-dose radiotherapy in combination with their nMOFs induces sufficiently potent immune responses to inhibit tumor growth in mouse models of colon and breast cancer.

Digitonin-Loaded Nanoscale Metal-Organic Framework for Mitochondria-Targeted Radiotherapy-Radiodynamic Therapy and Disulfidptosis | *Advanced Materials*, 2024 September 10



Spencer Watson

Immunofluorescence image of tumor cells just at the moment of tumor recurrence. Tumor cells in green appear to be streaming out of red areas of fibrotic scarring that protected them after treatment.

# The scars of therapy seed GBM resurgence

A study led by Ludwig Lausanne's Johanna Joyce and alumni Spencer Watson and Anoek Zomer and featured on the cover of *Cancer Cell* in September reported that recurrent glioblastoma multiforme (GBM) tumors can grow out of the fibrous scars caused by the treatment of the initial tumor in mice as well as humans. Studies in mice revealed that the scars create a niche that shields residual glioma cells from immunosurveillance and pushes them into a dormant state in which they resist therapy. The team integrated several technologies—including single cell gene-expression analysis, proteomics and an AI-powered workflow of analytical methods for the spatial analysis of tissues developed by Spencer in Johanna's lab—to analyze the cellular and molecular geography of the scars and resurgent tumors. They discovered that cells associated with tumor-feeding blood vessels become functionally altered to resemble fibroblasts. These perivascular-derived fibroblast-like (PDFL) cells fan out across the region previously occupied by the regressing tumor, where they mediate the generation of fibrotic scars. They found that PDFLs are activated by neuroinflammation and immune factors known as cytokines, especially TGF-β. The researchers devised a treatment regimen using existing drugs to block TGF-β signaling and suppress neuroinflammation in combination with CSF-1R inhibition and showed that it inhibited fibrotic scarring, diminished the numbers of surviving tumor cells and extended survival in a mouse model of GBM.

Johanna Joyce

Fibrotic response to anti-CSF-1R therapy potentiates glioblastoma recurrence | *Cancer Cell*, 2024 September 9

# Drug-induced sugar high boosts T cell efficacy in immunotherapy

Cancer cells favor a process for extracting energy from sugar known as glycolysis that most healthy cells only deploy when oxygen is in short supply. This phenomenon—the Warburg effect—depletes glucose in the tumor microenvironment, depriving anti-tumor T cells of a nutrient they too require to function effectively. Targeting cancer cell glycolysis would be a great way to improve immunotherapy, but it must be done without compromising T cell functionality. One candidate for such targeting is an enzyme called lactate dehydrogenase (LDH) that plays a key role in glycolysis and is expressed at much higher levels in tumor cells than in T cells. Researchers led by Jedd Wolchok and Taha Merghoub, co-directors of the Ludwig Collaborative Laboratory at Weill Cornell, Ludwig MSK alumnus Roberta Zappasodi and first-author Svena Verma reported in *The Journal of Clinical Investigation* in September that treatment with an LDH inhibitor decreases tumor cell glucose uptake and proliferation but has exactly the opposite effect on tumor-infiltrating T cells in mice. The treatment pumps up glucose levels in the microenvironment, enhances T cell killing of cancer cells and inhibits immunosuppressive regulatory T cells (Tregs) in culture. Combined with immune checkpoint blockade therapy, it effectively controls melanoma and colon cancer progression in mice by boosting effector T cell infiltration and activation while destabilizing Tregs—suggesting a new combination therapy.

**Pharmacologic LDH inhibition redirects intratumoral glucose uptake and improves antitumor immunity in solid tumor models** | *The Journal of Clinical Investigation*, 2024 September 3

Jedd Wolchok

Taha Merghoub

Shibin Zhou

Bert Vogelstein

# A Co-STAR for the tumor-targeting show

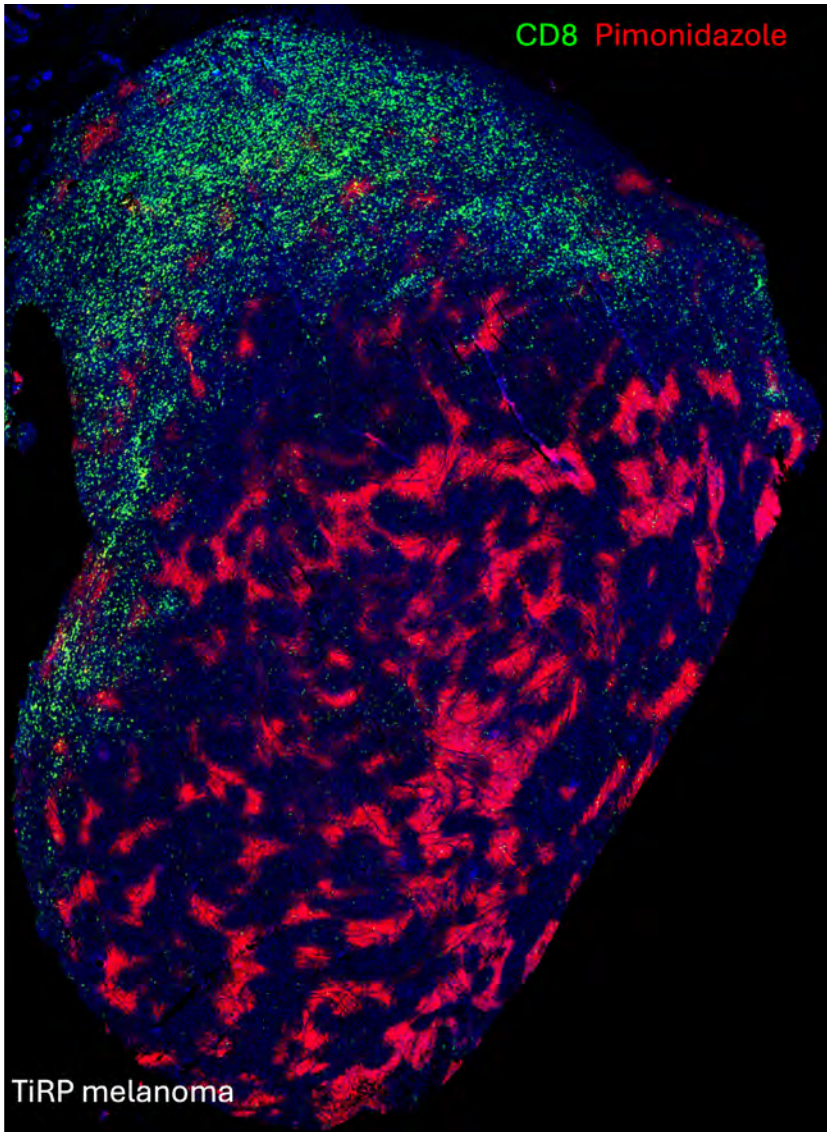
Each of the two primary modes of T cell engineering used for cancer therapy—chimeric antigen receptors (CARs) and engineered T cell receptors (TCRs)—has its limitations. CARs, which use the antigen-binding domains of antibodies, have a high affinity for their target antigens but require a lot of antigens to be present on their target cells to function effectively. TCRs, meanwhile, detect low levels of antigens, but also have low affinity for their targets. Researchers led by Ludwig Johns Hopkins' Shibin Zhou and Co-director Bert Vogelstein reported in a July issue of *Science Translational Medicine* their development of cells equipped with an engineered receptor they call costimulatory synthetic TCR and antigen receptor (or Co-STAR) that combines key elements of both CARs and TCRs. The antigen-recognizing elements of TCRs are replaced in Co-STARs by high-affinity antibody fragments, and costimulatory activating signals within the cell are provided by two modules that drive NF-κB signaling (MyD88 and CD40). Shibin, Bert and their colleagues designed a Co-STAR to target a common p53 neoantigen and showed it kills cancer cells in culture that present very low levels of the neoantigen. They also demonstrated that Co-STAR-bearing T cells expand better and induce more durable tumor regressions in mouse models than T cells equipped with similarly targeted TCRs and the same costimulatory molecules.

**Preclinical studies show that Co-STARs combine the advantages of chimeric antigen and T cell receptors for the treatment of tumors with low antigen densities** | *Science Translational Medicine*, 2024 July 10

# A stabilized T cell hypoxia response improves adoptive cell therapy

The harsh, oxygen-starved microenvironment of solid tumors can pose an insurmountable challenge to antitumor immunity and immunotherapy, often pushing T cells of the immune system into a dysfunctional state known as exhaustion and inhibiting T cell infiltration (see image). Researchers led by Ludwig Institute's Jingjing Zhu and Benoît Van den Eynde explored how the stabilization of the cellular oxygen sensor—hypoxia inducible factor (HIF)—would affect the antitumor activity of T cells used for adoptive cell therapy (ACT) in mouse models of cancer. The HIFs, which switch on a host of genes that help cells adapt to oxygen starvation, are known to play an important role in regulating T cell metabolism and function. Jingjing, Benoît and colleagues reported in a September issue of *Nature Communications* that activated tumor-targeting T cells used for ACT were far more effective when genes encoding PHD2 and PHD3, enzymes that help degrade HIF to regulate the hypoxic response, were selectively deleted using CRISPR-Cas9 gene editing. This selective deletion stabilizes HIF-1α signaling and boosts glycolysis, a metabolic process for harvesting energy from sugar that is critical to the function of activated T cells. When used for ACT, T cells lacking PHD2/3 induced potent therapeutic responses against several mouse models of cancer, including those that typically resist immunotherapy. The findings suggest new strategies to improve the efficacy and applicability of ACT for cancer therapy.

Enhanced tumor response to adoptive T cell therapy with PHD2/3-deficient CD8 T cells | *Nature Communications*, 2024 September 6




Benoît Van den Eynde laboratory

A micrograph of a melanoma tumor from the TiRP mouse model, which was used for this study. The staining shows CD8+ T cells in green, and a marker for cells exposed to hypoxia, named pimonidazole, in red. Notice how CD8+ T cells fail to infiltrate hypoxic areas of the tumor.

Benoît Van den Eynde

Jingjing Zhu

# Tumor Aneuploidy: A predictive biomarker of response to immunotherapy

Recent studies have linked elevated tumor aneuploidy, or abnormal numbers of chromosomes in cancer cells, to the suppression of anti-tumor immune responses and poorer prognosis following immunotherapy. Aneuploidy has been shown to suppress the infiltration of CD8+ T cells and natural killer cells into tumors and compromise their function. Ludwig Chicago's Sean Pitroda and a University of Chicago colleague explored whether aneuploidy scores could serve as prognostic markers of response to immune checkpoint blockade (ICB) in patients with non-small cell lung cancer (NSCLC). Analyzing data from a 309-patient cohort in which Sean's team linked aneuploidy to poor ICB response, they further demonstrated a novel association between elevated aneuploidy and nonsmoking-associated NSCLC oncogenic driver mutations, validating their findings in an independent cohort of 350 NSCLC patients treated with ICB. They reported in their August paper in *Scientific Reports* an association of high aneuploidy with immunosuppressive tumor states and focal amplifications of the TERT gene. Aneuploidy scores exhibit a dose–response relationship with objective response rate and overall survival. Further, patients with aneuploidy affecting more than half of the genome were especially unlikely to exhibit an objective response to immunotherapy. Aneuploidy's negative effect on survival was, however, attenuated by combination anti-CTLA-4 and anti-PD-1 antibodies in comparison to single-agent therapy. Their findings underscore a potentially useful role for measures of tumor aneuploidy in guiding immunotherapy.

Sean Pitroda

**Clinical and molecular correlates of tumor aneuploidy in metastatic non-small cell lung cancer**
*Scientific Reports*, 2024 August 21

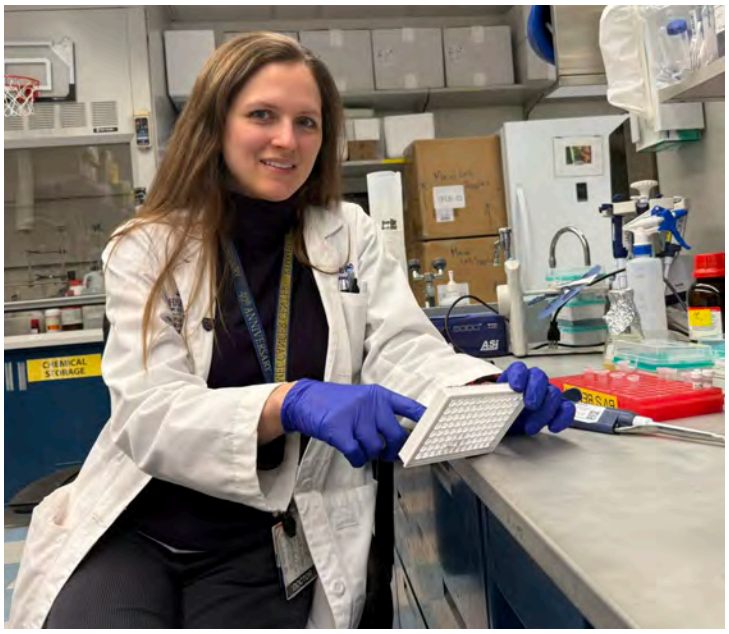
LUDWIG'S EARLY-CAREER RESEARCHERS

# Into a life of science

Three Ludwig Center trainees took a few minutes out of their very busy schedules to tell us about their lives, scientific fascinations, avocational passions and views on a variety of issues.

**Jacqueline Douglass**

Medical Oncology Fellow
Ludwig Johns Hopkins



**Jenny Hogstrom**

Postdoctoral Research Fellow
Ludwig Harvard

**Quenton Rashawn Bubb**

MD-PhD Candidate
Ludwig Stanford

# Quenton Rashawn Bubb

MD-PhD Candidate, Ludwig Stanford

**Tell us about yourself, where you come from, where you were raised, your family.**

I was born in Brooklyn, New York. I'm a child of immigrants from the island of Grenada. My parents met when they were very young in the church community in St. David's, Grenada. They immigrated to the United States in the early to mid '80s. My mom was a registered nurse, so she was in nursing in Brooklyn for pretty much the entirety of her career. My dad is an electrician. We had a really interesting upbringing because my mom would be working nights sometimes and would sleep during the day. My dad would pick us up from school, and on a week-to-week basis she had different shift schedules. We would oftentimes sort of have to improvise as far as who our main caretaker was. They were a pretty unique team for me and my brother.

**Where did you go to college and what was your major?**

I went to Johns Hopkins University, in Baltimore, where I studied biophysics. While I was in high school, I had this major dilemma. I knew that I wanted to somehow be in the periphery of medicine, and biology was really fun and cool, but I fell in love with math and physics. At that time, I didn't really know that engineering was an option, but when I was Googling "biology and physics programs," Johns Hopkins Biophysics came up, and I was like, "Oh my God, I could do biology and physics at the same time!" So that's what I really got into in college. Johns Hopkins was a very protein and structure-based program. That was my first deep dive into that sort of thing. It was tough, but also extremely gratifying.

**Do you feel like your biophysics background has helped you?**

Yeah, absolutely. For my PhD project, I set this ambitious goal to create a CAR T cell that can target three different antigens simultaneously through one CAR receptor. So I had to ask very fundamental questions about what kinds of proteins one should utilize in creating this kind of thing. One issue with using antibody or scFvs to make a trivalent receptor is that they aggregate. They have really sticky hydrophobic surfaces that can make it difficult for them to express. Early in my PhD, I had this wacky idea that, instead of using scFvs, what if we just use the ligands of the proteins that we are targeting. Here you have a folded unit, a globular unit that can fold independently of other globular units, that are attached via linkers and express extremely well. I consider that to be a triumph of my biophysics background and understanding, having intuition about folds for that earlier design.

**What led you specifically to cancer research?**

I think I took a sort of winding path toward cancer research. I mentioned that I'm a child of immigrants from the island of Grenada and one of the first characterizations of sickle cell disease came from the island of Grenada. Many people in my family had sickle cell disease, so it had always been on the back of my mind. While I was in college, I learned that Linus Pauling wrote a paper on sickle cell disease and put forth some elegant analyses and hypotheses about how the disease works at a molecular level. That I would say is the first time hematology entered my mind's eye. I then received the Marshall scholarship, and I completed a one-year program studying protein folding at the University of Cambridge. Around that time, I learned about potential cures for sickle cell disease, which included gene therapies. For

> "I'm oriented toward becoming a physician-scientist. The kind of physician-scientist that I want to be is the hard question right now."

gene therapies to take, you have to get rid of a patient's immune system to replace it with a new one. That includes chemotherapy and radiation, and I initially thought that was ridiculous. Why would a person, who's dealt with these crazy pain episodes their entire life, opt into irradiating their bodies to get a potential cure? Around then, I was exposed to the research of my PhD advisor Agnieszka Czechowicz, who was exploring ways to establish transplants without having to use chemotherapy or radiation. In settings like sickle cell disease, not using chemotherapy or radiation has potential to significantly improve access to cure.

When I got started at Stanford for my MD-PhD after Cambridge, I learned that this transplant problem is also a significant barrier for malignant hematopoietic diseases like acute lymphoblastic leukemia and acute myeloid leukemia. Patients go through this "conditioning process" where they get chemo and/or radiation to clear out their blood system, and then they get a stem cell transplant to give their body a new immune system to add an additional layer of protection against that leukemia. In some disease settings, a significant proportion of the mortality that you see from stem cell transplantation comes from the conditioning itself.

This idea that came together was a collaboration between my main advisor Agnieszka Czechowicz and a co-advisor, Crystal Mackall, to create a cell therapy that

can go into the person, destroy malignant disease, destroy the person's healthy or pre-leukemic, hematopoietic system as a two-in-one. They could get a potentially curative transplant without chemotherapy or radiation, which I thought was a pretty clever approach.

**Any avocational interests, hobbies?**

I'm one of the biggest jazz nerds that you'll ever meet. I've been into music since I was a young child. My dad is a guitar player and saw it as important for me and my brother to understand our musical selves. So I've been playing piano since I was four years old, and I started playing the drums in high school along with many other interesting instruments. I started playing the harp in high school as well. It's hard to come by a harp these days, so I haven't played it since.

In high school, I started playing jazz music and it has stuck. I consider myself a professional amateur, in the sense that I'm not paid to make jazz music, but I still make it anyway. I'm an avid listener and I try to see as many live shows as possible. So if I'm not in the hospital, if I'm not in the lab, if I'm not playing video games, I'm probably listening to jazz music or making it.

**Who are your favorite jazz musicians?**

That is tough. When I'm asked this question I always separate it into people who are alive and people who are no longer here. So right now, my favorite musician is Jaki Byard, a piano player who became a master of all the styles of piano. In the middle of my PhD my mom passed away from a uterine cancer. It was tough, as she was in New York, and I was in California. But, around that time, I discovered Jaki Byard, and he has this album called Parisian Solos that I probably listened to more than 100 times in the year that she got sick. It's like a tour de force of Jaki Byard's virtuosity. He has these really emotional and experimental ideas and, at least to me, the album represents the ways in which life in its simplest way, can have a lot of rhythm to it. It helped me really lock in on a day-to-day basis, which I think is really important when you're supporting family through crises like that.

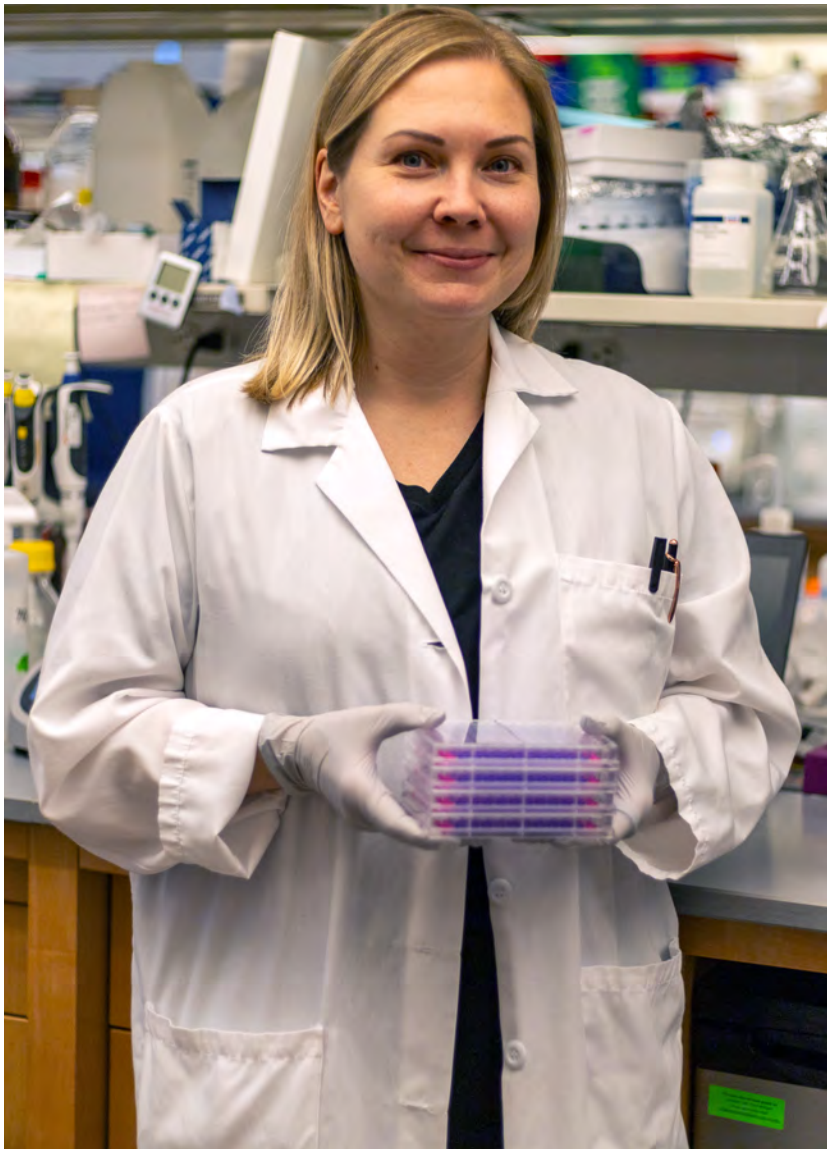
And as far as people who are alive right now, ask me an instrument and I'll tell you my favorite player, but I would say that there's a drummer named Marcus Gilmore who I think is one of the best drummers alive right now. He plays out in New York and he makes some really awesome music as well.

**What do you want to do next? Do you want to focus on research? Do you want to be a physician-scientist? What are you thinking?**

This is the hard question. I'm oriented toward becoming a physician-scientist. The kind of physician-scientist that I want to be is the hard question right now. I hope to enter a residency program that's physician-scientist focused, and it'll likely be in internal medicine, toward hematology/oncology. I'm still brainstorming about whether or not my value is in a lab, in the hospital, in biotech, or a mixture of the three.

So, in a very jazzy sense, I'm improvising, taking it one step at a time. A sort of surprising realization that I had in the last year, I think, is that I've always thought about being a physician-scientist in terms of what I could produce, what can I discover, what therapies can I create, et cetera. I didn't really think as much about the actual facilitation part. How much can I grow scientists to think, create, and discover? That idea has actually become more valuable to me over time. The idea of not only just having a lab that's working on basic and translational questions, but also having a lab in which I'm fostering young minds and helping them discover.

This has been one of the reasons why I've thought seriously about running a lab. But we'll see how things pan out and how my priorities change as I proceed through my training.



# Jenny Hogstrom

Postdoctoral Research Fellow, Ludwig Harvard

**Tell us a bit about yourself, where you're from, where you were raised, and a bit about your family.**

I'm from Finland and I did all my education there, so bachelor's, master's and PhD at the University of Helsinki. I was the first in my family to go to university. I was always fascinated by biology, ever since I was in elementary school. In high school I got very interested in genetics, and at that point I started thinking maybe this could be something that I would like to study. And my parents, although they have no background in science and are from working class families, they were very encouraging that I should go to university and study what I am passionate about. I'm also very lucky that Finland has free education. But it was really at the masters stage that I became interested in cancer research. I had to pick a subject to start research, and there were a lot of interesting cancer labs at University of Helsinki. I ended

"I work with hormone receptor-positive breast cancer and mainly from a tumor microenvironment point of view."

up studying colorectal cancer and patient-derived organoid models, and that's what I did my thesis on.

**What do your parents do?**

My mom works for the police. She worked first as a secretary and then dealing with driving licenses. My dad, he's been a chef, then he went into real estate and selling furniture. So very far from what I'm doing.

**Do you have any siblings?**

I do. I have a younger brother and a much younger sister. They're back in Finland. My whole family is there.

**What is your research focused on now? What scientific challenges is your work looking to address?**

I work with hormone receptor-positive breast cancer and mainly from a tumor microenvironment point of view—so, specifically, the cancer-associated fibroblasts and what they secrete to make these breast cancer cells resistant to targeted therapies. I'm also especially interested in patient-derived models, so I have established matching cancer-associated fibroblasts and patient-derived organoid cultures from patient biopsies of both primary and metastatic breast cancer.

**What interests you about that subject, specifically about the patient models?**

We really need better models to study hormone receptor-positive breast cancer. We wanted to try to see if we could do it from biopsies, and if we could derive both stromal cells from the tumor microenvironment with the breast cancer cells, and we were successful in that. Now we're using these models to study drug resistance to targeted therapies.

Of course, it's not complete—we don't have immune cells—but we do have the cancer-associated fibroblasts, and we are interested in what they are secreting and how that stimulates drug resistance. I'm personally very interested in cell metabolism, so I'm investigating what metabolites they are secreting and how that affects the breast cancer cells and drug resistance. And this is something that hasn't really been studied that much, especially not in breast cancer

and hormone receptor-positive breast cancer.

**What emerging technologies in your field excite you the most?**

Well, I have a certain technology that I'm very excited about, which is the single-cell metabolomics. It's on the way. That's something that excites me a lot because then we can look at how metabolism is different in different cells. The tumor microenvironment and breast cancer cells are very heterogeneous, and I can only imagine that metabolism in these different populations is very different. So I'm hoping that will be available in the next five years.

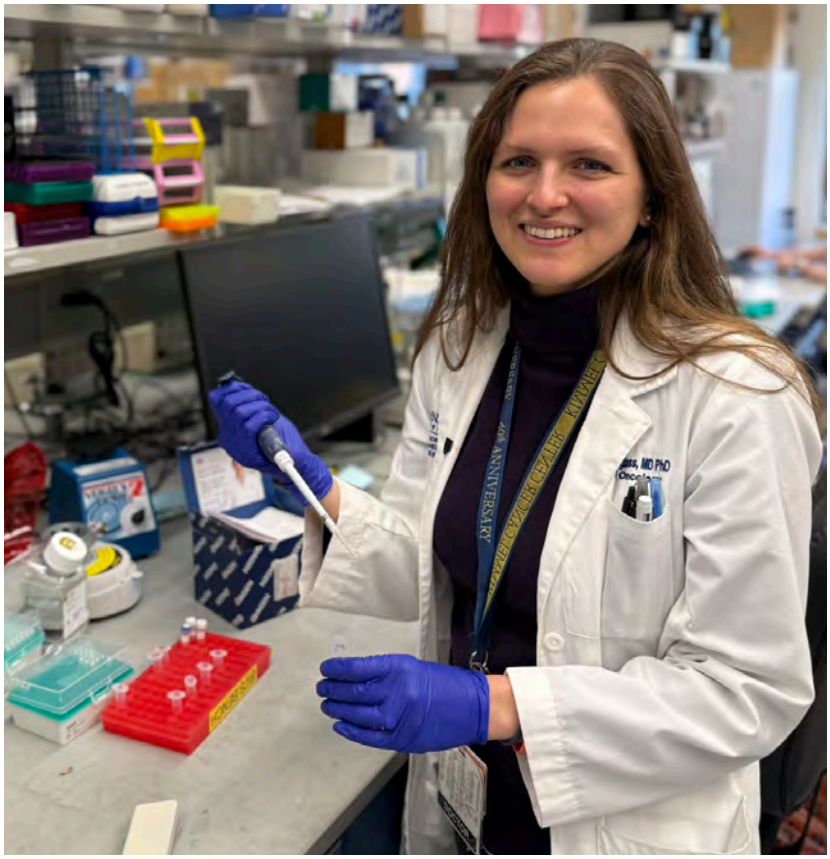
**What are your hobbies?**

I have a dog, so at least when he was younger, we were very interested in different types of training, obedience training, of course, spending time with him. He is a Labrador. They're very smart and very good at learning stuff. So that's what I was doing most of the time. I mean, I love traveling also but I'm getting to do that less and less. I wish I had more time, because I used to travel a lot when I was younger and just love seeing new places.

I'm also doing renovations at the moment, so that's my free time. Learning new things that I never thought I would.

**Is there anything you do to incorporate Finnish holiday traditions over here?**

I try to incorporate some of the foods. I love the Finnish Christmas pastries. It's just a puff pastry with, like, plum jam. Oh, that's my favorite. That's something I can make here. Then all of the other ones are something that my parents always made, and I should probably learn how to make all of those dishes.



# Jacqueline Douglass

Medical Oncology Fellow, Ludwig Johns Hopkins

**Could you tell us a bit about yourself, your educational background, and how you got into medicine?**

I was born in Charleston, South Carolina, but I spent most of my childhood in Memphis, Tennessee. Both of my parents were in the Air Force, so when I was younger, we moved around quite a bit. My dad studied electrical engineering and worked as an Air Force pilot then commercial airline pilot. And my mom

"In science, it's important not to overcommit to too many things and instead focus on just a couple projects so that, ideally, you can see those to fruition. I tend to be the type of person who gets excited about new ideas and novel projects, but I find it easy to become overextended."

trained as a pharmacist and a dentist. I have one younger sister who now works in tech. Math and science were my favorite subjects in grade school. I went to undergrad at MIT, where I had diverse interests, but ultimately decided to double-major in chemical-biological engineering and economics. I didn't know what career I wanted to pursue for most of my undergrad. I had interned in finance, including at Lehman Brothers in 2008. I ultimately decided against this career path, not so much because of the market crash but because I felt that the people I worked with in finance did not seem very fulfilled by their jobs.

When I came back for my senior year at MIT, I had to take one more biology class to complete my degree requirements. I had two excellent professors teaching this biology class, and they seemed so happy and enthusiastic about what they were teaching, which was a refreshing change from what I had observed in finance. I realized I needed to reconsider my career path. I had done lab research earlier in undergrad, and I decided to get involved in research again which made me remember how much I enjoyed wet lab work. Additionally, during my senior year, I was able to shadow a radiation oncologist, which was a pivotal experience. I felt that he had an incredible job being able to care for and guide cancer patients while at the same time working closely with physicists and other physicians, and using sophisticated machines to provide treatment to patients. Based on these experiences, I ultimately decided to pursue a career as a physician-scientist.

After undergrad, I stayed at MIT an extra year to get a master's degree in chemical engineering. And then I spent a year at the NIH in the Intramural Research Training Award program, where I did full-time research for a year while applying to MD/PhD programs. I ultimately did my MD/PhD at Johns Hopkins, where I also trained in internal medicine. I'm currently a medical oncology fellow in my second year, and I'm back in the same lab as my PhD lab, doing similar research.

**Tell us about your research ...**

All nucleated cells in the body express peptide-HLA complexes on their surface as a way for the immune system, and specifically T cells, to sample the contents of those cells. This is a way for T cells to know which cells to kill. So, for example, a virally infected cell will display viral peptides on the cell surface and invite T cell-directed attack. In cancer cells, mutant peptides derived from mutant proteins can be displayed by HLA on the cancer cell surface. These mutant peptide-HLA complexes are potentially an ideal target of therapy as they are a truly cancer-specific, cell surface marker.

Peptide-HLAs are typically a ligand for T cell receptors (or TCRs). However, antibodies have some distinct advantages over TCRs, so we are trying to make antibodies that mimic

T cell receptors, in that they specifically bind to these mutant peptide-HLA complexes. Then when we identify a given antibody, we can transform it into various T cell-based therapeutic agents such as bispecific antibodies or CAR-T cells. Specifically, we are interested in targeting common mutations found in what are called driver genes (that is, oncogenes or tumor suppressor proteins), as these same mutations are found in many cancer patients and the cancer cells depend on the presence of these mutations for their tumorigenicity. The ultimate goal is to create a panel of these antibodies that would be grafted into an optimized therapeutic format and then could be used in an off-the-shelf manner to treat patients with one of many cancer types.

**What interests you about this subject?**

I find this an exciting project because it presents major challenges but has the potential to be useful essentially for all cancer types. Nearly all cancer cells contain mutations in their genome, so this strategy could be a completely novel class of anti-cancer drugs, one that is broadly applicable.

**How does your background in economics play into your research when you think about things like accessibility?**

As a physician when I'm caring for a patient, my goal is to pick the safest and most effective therapy for my patient. However, my economics background does make me concerned about the accessibility of these therapies, as well as the ability of our healthcare system to shoulder the costs of such expensive novel therapies. For example, from a societal perspective, can we afford to give every cancer patient a $500,000 CAR T-cell therapy or a $200,000 course of bispecific antibodies? There's a big price tag on these novel immunotherapies, and I think we as a society will have to think about whether our healthcare system can afford such therapies. I am, however, optimistic that as these therapies become more ubiquitous, with manufacturing optimization and economies of scale, we'll be able to bring down their costs substantially.

**What are your favorite avocational activities?**

As far as hobbies, I'm married and I have two young kids, a two-and-a-half-year-old son and a three-and-a-half-month-old son. My husband is a resident in a surgical field who works even longer hours than I do. So, currently, my main hobby, if you would call it that, is spending time with my sons. But I also really enjoy physical fitness. I like cycling and running, and at least once a week I try to attend this hot yoga class called yoga sculpt that involves cardio and weights and music. The class is really fun and an excellent workout. I think it's important to have time to myself to focus on my physical and mental fitness.

**What would be your advice to young women who are trying to decide what they want to do and are maybe worried about not being able to have a family and be a successful physician-scientist?**

I'm not going to lie, it's tough. When I was in my 20s, I didn't make career decisions based on perceived future limitations from having a family because it seemed so far in the future. I also underestimated the time-commitment that comes with having kids and running a household. Now, my advice would be that if having children is a priority for you, it does require significant sacrifice but that shouldn't be a deterrent. The sacrifice comes in several forms—financially, for childcare expenses, time-wise, in caring for them or doing extra childcare related chores, and, to some degree, career-wise because it is very difficult for both parents to have high-powered careers in academic medicine while permitting one of them to be available for daycare drop off/pick up, dinner time, doctor's appointments, sick days, etcetera. Picking a partner who will share responsibilities is critical. Also, getting help from family if you can is a big plus. For the first two years of my older son's life, while my husband and I were both in residency and often both working overnight shifts, our moms took turns staying with us to watch him, which was a tremendous help. I do anticipate things will get easier as they get older and are more independent, so this time when they are little and need so much is temporary. One piece of advice I've heard is that you should make family planning decisions around what you want your Thanksgiving table to look like in 20-30 years, which I think is wise. Additionally, the level of joy I get from my sons is incomparable to anything else. It's a lot of work, but anything that's important and meaningful is going to require effort and sacrifice and that's very much the case when having kids.

**What's the best career advice you've received?**

I think the best advice is something that Bert Vogelstein said, which was something to the effect that "ideas are cheap, but your time and focus are very valuable." In science, it's important not to overcommit to too many things and instead focus on just a couple projects so that, ideally, you can see those to fruition. I tend to be the type of person who gets excited about new ideas and novel projects, but I find it easy to become overextended. While I do think it's important for trainees to hedge their bets by having a couple projects, perhaps something safer and something riskier, if you have too much going on, you won't be able to think deeply about any one problem.

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